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Protein and Cholesterol Levels of Duck Eggs after the Addition of Nanochitosan as a Feed Additive

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Abstract. Nanochitosan is a polysaccharide that can be used as a feed additive to improve the chemical quality of eggs. Protein and cholesterol levels are indicators of the chemical quality of eggs that affect egg weight. This study aims to analyze the effect of nanochitosan as a feed additive on improving the chemical quality of eggs in terms of protein content, cholesterol content, egg white weight, egg yolk weight, and duck egg weight. This study was designed in a completely randomized design (CRD) consisting of 5 treatments (0; 2.5; 5; 7.5; 10 g nanochitosan/kg feed) with 5 replications. Treatment of feed was given for 8 weeks. Variables measured included protein content, cholesterol level, egg white weight, egg yolk weight, and duck egg weight. Data was analyzed using ANOVA at 5% significance. The results showed that nanochitosan as a feed additive had a significant effect on increasing protein content, egg white weight, egg yolk weight, egg weight and cholesterol reduction. The conclusion of this study is that nanochitosan can be used as a feed additive that has potential to improve the chemical quality of duck eggs. The novelty of this research is the use of nanochitosan as a feed additive to improve the performance of livestock in increasing protein levels and reducing cholesterol levels in pengging duck eggs. The results of this study are expected to open up opportunities regarding the use of nanochitosan as a feed additive in various other local ducks in Indonesia.

Keywords: cholesterol; feed additive; nanochitosan; pengging duck eggs; protein

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INTRODUCTION

People's need for animal protein from year to year has increased. One of the animal proteins utilized by Indonesian people is duck. The advantage of duck farming is that ducks are adaptable and not easily stressed (Ismoyowati *et al*., 2019). Duck eggs are one of the sources of animal protein needed by the body which contain various kinds of protein and essential amino acids (Sulaiman *et al*., 2023).

It is known that the protein content of pengging duck eggs is lower than the eggs of other ducks. The average egg protein content of pengging ducks is 9.24%, while that of Tegal ducks is an average of 10.74% (Mahfuz & Piao., 2020). The weakness of duck eggs is that they have high cholesterol levels. Ismoyowati *et al*. (2022) stated that the eggs of Pengging ducks had higher cholesterol levels than the eggs of Magelang and Tegal ducks. Cholesterol in the eggs of wading ducks, Magelang ducks, and Tegal ducks, respectively 921.57; 913.28; and 827.81 mg/100g. Protein and cholesterol in eggs can affect egg weight. Ahmad *et al*. (2018) reported that egg white weight can be affected by the presence of protein in the egg and egg yolk weight is affected by the presence of protein and cholesterol.

The chemical content of eggs in the form of protein and cholesterol levels in the eggs can be caused by the quality of the feed, digestive processes, and metabolism (Rossida *et al*., 2019). Adding additives to feed can be done to improve feed quality and livestock performance. Feed additives are additional feed ingredients given to livestock which function to improve the quality of livestock production and regulate the rate of absorption of nutrients (Sunarno *et al*., 2021). The use of antibiotics in animal feed has been banned in Indonesia because they can cause residues in tissues which result in contamination through the food chain (Pasaribu, 2019). The addition of antibiotics as feed additives is still not effective. Another effort that breeders can do is to use feed additives made from natural, safe, inexpensive, and high benefits (Ayman *et al*., 2022). One of the feed additives that meet these criteria is nanochitosan.

Nanochitosan is a polysaccharide that can be synthesized from shrimp, crabs, and lobsters that contain chitin (Rumengan *et al*., 2017).

Nanochitosan has an amine group (-NH_2) and a hydroxyl group (-OH) which are polycationic which are reactive with the surrounding negative ions (Nuengjamnong & Angkanaporn, 2017). Nanochitosan is able to bind negatively charged cholesterol thereby reducing cholesterol absorption in the digestive tract. The complex bond in nanochitosan to cholesterol will be wasted along with the feces (Tufan & Arslan, 2020). Research conducted by El-Naby *et al*. (2019) proved that providing nanochitosan in feed with level of 1, 3, 5 g/kg of feed could increase growth, total feed consumption, and total serum protein in Japanese quail. El-Ashram *et al*. (2020) stated that giving nanochitosan to birds, broiler chickens, and laying hens as a feed additive with concentrations below 1.4 g/kg BW per day is safe and has the effect of lowering cholesterol and free fatty acids in poultry serum. Sunarno *et al*. (2021) reported that the addition of nanochitosan as a feed additive 2.5; 5; 7.5; and 10 g/kg for 8 weeks had an effect on increasing nutrient absorption in Tegal ducks.

The use of nanochitosan as a feed additive has not provided much information regarding protein content, cholesterol, egg white weight, egg yolk weight, and egg weight in wading ducks. Based on this, this research was conducted to analyze the levels of protein, cholesterol, egg white weight, egg yolk weight, and egg weight in pengging ducks which were given nanochitosan as a feed additive.

This research is an attempt to obtain high quality chemical quality duck eggs for consumption purposes and to analyze nanochitosan feed additives in improving digestive and reproductive performance as reflected in increased protein content, egg white weight, egg yolk weight, egg weight and decreased egg cholesterol levels.The data from this study are very useful as a reference in administering nanochitosan as a feed additive to local ducks reared by breeders in Indonesia

METHODS

Research Plan

The research was conducted at the People's Farm, Bawak Village, Cawas, Klaten. Chemical Quality Analysis of duck eggs was carried out at the Wahana Semarang Laboratory. This study used a completely randomized design (CRD) with 5 treatments and 5 replications. The treatment was in the form of nanochitosan addition in feed P0 with a level of 0 g/kg feed (control); P1; P2; P3; P4 with nanochitosan content of 2.5 g/kg feed (standard feed + 2.5 g/kg feed); 5g/kg feed (standard feed + 5 g/kg feed), 7.5 g/kg feed (standard feed + 7.5 g/kg feed), and 10 g/kg feed (standard feed + 10 g/kg feed). The treatment was carried out for 8 weeks. The research variables of the bucking ducks consisted of nanochitosan as the independent variable and protein content of egg white, egg yolk cholesterol, egg white weight, egg yolk weight, and egg white weight of ducks as the dependent variable. The control variables measured were temperature and humidity in the cage.

Making Feed Formulations with Nanochitosan Additives

The addition of nanochitosan into the feed is carried out in a way, namely nanochitosan with a predetermined level is added to every 1 kg of standard feed weight and stirred until it is homogeneous. Homogeneous feed is weighed as much as 400 g which will be given to 5 ducks. The feed given to the ducks was in the form of semi-wet mash (400 g of standard dry feed mixed with 135 ml of water/400 g of feed) which had been mixed with nanochitosan (Sunarno *et al*., 2021).

Preparations and Acclimation of Test Animals

The experimental animals used in this study were 25 female 5-month-old ducks, ducks in healthy condition with body weights ranging from 1.1 kg to 1.4 kg, having entered sexual maturity or in the production period. Healthy ducks can be identified based on their characteristics, namely the ducks have dull feathers, clear eyes, no slimy noses, and are active. Pengging ducks were placed in 5 plots of cages, each plot measuring 100×150×70 cm^3. Each cage plot contains five pengging ducks. The partition between the cage plots was in the form of bamboo slats and the lining of the cage was in the form of rice husk litter. Each cage plot was equipped with a place to feed and a place to drink water. Prior to feed treatment with the addition of nanochitosan additive, the ducks were acclimated for one week to adapt to the new environment. Feed is given twice a day, namely in the morning at 08.00 WIB and in the afternoon at 16.00 WIB. Drinks were given ad libitum.

Research sampling and Measurement

Duck egg sampling was carried out every morning from each cage plot for 28 days starting from week 5 to week 8 followed by measurement and analysis of research variables. Each duck egg was marked with a marker, namely the type of treatment and the date of collection. Measurement of egg weight, egg white weight, and egg yolk weight was weighed using a digital scale. Egg white protein levels were measured using the Kjeldahl method (Mori *et al*., 2020). Egg yolk cholesterol levels were measured using the Liebermann Burchard method (Ukachukwu,*et al*., 2017).

Data Analysis

The data obtained from this study were analyzed to obtain distribution patterns and homogeneity. Test for normality using the Test of Shapiro-Wilk. The results of data analysis showed a normal and homogeneous distribution followed by analysis using Analysis of Variance (ANOVA) with a significance level of 5%. The ANOVA results which show a significant difference are followed by the Duncan Multi Range Test at a significance level of 5% using the SPSS (Statistical Product of Service Solution) application for Windows version 26.0 (Directorate General of Animal Husbandry and Health, 2021).

RESULT AND DISCUSSION

ANOVA results showed that there were significant differences in protein content, cholesterol level, egg white weight, egg yolk weight, and total egg weight between the treatment and the control (P<0.05). The research data showed that the addition of nanochitosan as a feed additive had an effect on protein content, which was indicated by an increase in egg protein content, a decrease in egg cholesterol, an increase in egg yolk weight, an increase in egg white weight, and an increase in total egg weight. Data from research on nanochitosan treatment as a feed additive are presented in Table 1.

Table 1. Average egg white protein, egg yolk cholesterol, egg white weight, yolk weight, and total egg weight of ducks after treatment with nanochitosan as a feed additive for 4 weeks

Variable	Nanochitosan treatment				
	P0	P1	P2	P3	P4
Egg white protein (%)	7.41^{a}±0.19	7.50^{a}±0.14	7.82^{b}±0.17	8.09^{c}±0.15	8.22^{c}±0.36
Egg yolk cholesterol (mg/100g)	432.65^{c}±9.19	402.46^{b}±8.02	408.25^{b}±7.05	367.21^{a}±3.61	357.54^{a}±13.15
Egg white weight (g)	30.02^{a}±0.45	31.50^{b}±1.37	32.78^{c}±0.67	33.86^{c}±0.57	36.16^{d}±0.73
Egg yolk weught (g)	19.74^{a}±0.51	19.93^{ab}±1.28	20.98^{b}±0.70	22.40^{c}±0.66	24.50^{d}±0.54
Total egg weight (g)	58.51^{a}±1.09	60.84^{b}±1.55	62.94^{c}±0.57	65.33^{d}±1.20	70.47^{e}±1.09

Note: The data in the table is the average ± standard deviation. Different superscripts located on the same line show significant differences (P<0.05). P0 (ducks fed standard feed without nanochitosan addition). P0, P1, P2, P3, and P4 respectively were ducks that were fed standard feed with nanochitosan addition of 0; 2.5; 5; 7.5; and 10 g/kg feed weight.

The results of data analysis using the ANOVA test showed that there was a significant difference in the average egg white protein content between treatments (P<0.05). Tests using the Duncan test showed that nanochitosan as a feed additive had an effect on increasing the protein content of pengging duck egg whites which could be seen in the P2, P3, and P4 treatments which were significantly different from the control. This shows that the addition of nanochitosan causes an increase in egg white protein levels in the duck eggs. The highest protein content occurred in P3 and P4 with concentrations of 7.5 and 10 g/kg feed weight. These microclimatic conditions are suitable for the life of laying ducks. The temperature and humidity in the laying duck cage were within the normal range. cage temperature ranged from 28-30^{0}C, while humidity ranged from 60-65%.

The average value of protein content in the egg whites of penging ducks in this study was smaller than the protein levels of duck egg whites conducted by Ganesan *et al*. (2014) that the protein levels of normal duck egg whites ranged from 8.60-10.50%. Low protein levels than normal can be caused by low absorption of feed nutrients used for egg production, so that the protein produced is low. This is in accordance with the opinion of Jiang *et al*. (2018) that nanochitosan increases the viscosity of gastric contents by forming a gel as a result of bonding with the surrounding negative molecules. This can delay gastric emptying, causing a feeling of satiety more quickly and limiting feed consumption.

Nanochitosan can increase egg white protein content due to the presence of cross-links between the amine groups of nanochitosan and the hydroxyl groups of the protein. This is in accordance with the statement of Rosyada *et al*. (2019) who showed that nanochitosan has an amine group (-NH_2) and a hydroxyl group (-OH). The functional groups contained in nanochitosan cause a cross-linking mechanism. Zou *et al*. (2016) states that the cross-linking that occurs between nanochitosan and protein involves an amine group (-NH_2) in nanochitosan with a hydroxyl group (-OH) in protein.

Nanochitosan cross-links can increase the catalytic activity of enzymes that play a role in helping protein digestion and egg formation. This is in accordance with the opinion of Laura *et al*. (2016)that nanochitosan is a complex polysaccharide that cannot be digested by the body, but can increase the catalytic activity of digestive enzymes. Vaghari *et al*. (2016) stated that immobilization of enzymes was carried out to facilitate the process of degradation of substrates by enzymes into products. Enzyme immobilization has several advantages, including the covalent bonds that have been formed which are not easily broken due to the influence of changes in pH, ionic strength, substrate, and high stability of the immobilized enzyme. Ali *et al*. (2016) stated that the activity of the immobilized enzyme is better than the free enzyme so that the bond formed can expand the surface of the enzyme thereby increasing the chances of attachment between the enzyme and the substrate. The immobilized enzyme can convert more products than the free enzyme at the same time. Xu *et al*. (2014) feed supplemented with low molecular weight nanochitosan can increase protease activity in the intestine.

Proteases are enzymes that play a role in hydrolyzing proteins into simpler ones, namely peptides and amino acids. Lu *et al*. (2016) stated that protein content greatly influences the process of egg formation. Amino acids are absorbed by enterocyte cells in the intestinal villi, circulated to the liver and then accumulate in the follicles of the ovaries which are carried by the bloodstream. The mature ovum is ready to enter the infundibulum and then enter the magnum as a place to secrete albumin.

The results of the statistical data analysis of the ANOVA test on the variable cholesterol level of the egg yolk of the rolling duck showed significant differences between the treatments ($P<0.05$). Duncan's test showed that nanochitosan as a feed additive had an effect on egg yolk cholesterol levels and this was evident in the P1 treatment with a concentration of 2.5 g/kg feed weight. The mean values of egg yolk cholesterol levels at P1, P2, P3, and P4 were significantly different and lower than the controls. Jiang *et al*. (2018), stated that nanochitosan is able to increase the viscosity of the contents in the stomach by forming a gel as a result of bonding with the negative molecules around it so that it can delay emptying in the stomach and ultimately cause a feeling of satiety more quickly and food consumption becomes limited. Nanochitosan content of 2.5; 5; 7.5; and 10 g/kg of feed weight has the potential to reduce cholesterol levels in pengging duck eggs. The highest cholesterol levels occurred in the treatment of nanochitosan 7.5 and 10 g/kg feed weight. The results of the study with the feed additive treatment of nanochitosan gave better results than the results of Rossida *et al*. (2019) with the treatment of moringa leaf flour feed additives (0, 2.5, 5, 7.5, 10%) which did not have a significant effect on reducing cholesterol in egg yolks in Pengging ducks.

Nanochitosan has a positively charged structure which is able to reduce cholesterol levels in pengging duck eggs by binding mechanisms. The mechanism of action of nanochitosan in lowering cholesterol levels is by inhibiting cholesterol absorption. This is in accordance with Li *et al*. (2016) who stated that nanochitosan is able to reduce cholesterol absorption in the intestine by binding to negative cholesterol molecules.

Decreased absorption of cholesterol in the intestine can cause a decrease in the level of cholesterol formed in eggs. Putri *et al*. (2020) stated that the formation of cholesterol in eggs begins when there is absorption of feed nutrients by enterocyte cells in the small intestine. The absorbed nutrients are then circulated through the veins in the intestine, then through the hepatic portal vein to the liver and then distributed through the blood vessels in the form of lipoproteins, then channeled into the follicles in the ovaries.

The results of the research on egg white weight variable using ANOVA analysis showed that there was a significant difference (P<0.05). Tests with the Duncan test on egg white weight showed that nanochitosan as a feed additive had an effect on egg white weight and this effect began to be seen in the P1 treatment with a concentration of 2.5 g/kg feed weight. The average egg white weight in the P1, P2, P3, and P4 treatments was significantly different and higher than the control. This condition indicates that the weight of egg white after administration of nanochitosan is 2.5; 5; 7.5; and 10 g has the potential to increase the white weight of duck egg compared to the control. The highest weight of white duck egg occurred in nanochitosan 5; 7.5, and 10 g/kg feed weight treatment. The results of the study with the feed additive treatment of nanochitosan gave better results than the results of Rossida *et al.* (2019) with the treatment of moringa leaf flour feed additives (0, 2.5, 5, 7.5, 10%) which did not have a significant effect on egg white weight in Pengging ducks.

Egg white weight can be affected by egg protein content. (Liu *et al*., 2020) states that protein is a factor that affects the viscosity of egg albumen, as the level of albumen viscosity increases, it will be followed by increasing egg weight. Nanochitosan can affect the quality of egg white by supporting protease activity in the process of protein digestion. Nurzamzam *et al*. (2015) states, the quality of egg white is largely influenced by the large amount of ovomucin secreted on the surface of the magnum. Ovomucin is the main ingredient that plays a role in the formation of foam and thickness in egg whites.

Ovomucin can be formed from protein sourced in feed ingredients when ducks consume feed. Zhu *et al*. (2015) stated that ovomucin is a glycoprotein in the form of a fiber which has the ability to bind fluids in eggs to be used as a gel structure in duck egg whites. This is in accordance with the statement of Rossida *et al*. (2019) egg white viscosity is formed from the interaction between ovomucin and lysozyme. Ovomucin has a function in binding water to form a gel so that the albumen will be thick. Lysozyme determines the level of thickness of duck egg whites with antibacterial properties. Ovomucin with lysozyme can cause a stable foam and increase the viscosity of the egg white so that the level of viscosity in the egg white becomes higher.

Analysis of the egg yolk weight variable using the ANOVA test showed that there were significant differences between treatments (P<0.05). Tests using the Duncan test showed that nanochitosan as a feed additive had an effect on egg yolk weight and began to show its effect on the P2 treatment with a content of 5 g/kg feed weight. The average weight of egg yolks in the P2, P3, and P4 treatments was significantly different and higher than the control. Nanochitosan as a feed additive at grade 5; 7.5; 10 g/kg has the potential to increase egg yolk weight.

Figure 1. Physical appearance of duck egg yolk after treatment with feed additive nanochitosan for 4 weeks

Hafeez *et al*. (2016) stated that the increase in egg yolk weight can be affected by cholesterol levels. Cholesterol levels contained in egg yolks can be affected by the absorption of cholesterol in the body. This study showed that cholesterol levels in egg yolk decreased after adding nanochitosan content to the feed. The increase in egg yolk weight is thought to occur due to the water content and increase in egg yolk protein. Lestari *et al*. (2015) stated that the results of amino acids that are absorbed by enterocyte cells in the intestinal villi are then circulated to the liver and then formed into protein. The protein formed will then be channeled to the ovary for the egg formation process. The availability of more protein will affect the increase in protein formed in eggs. Li *et al*. (2023) stated that nanochitosan is a cationic polymer that is insoluble in water, so that the water content can run normally during the egg formation process.

The results of the research on total egg weight variable using ANOVA analysis showed that there were significant differences between treatments (P<0.05). Tests using the Duncan test showed that nanochitosan as a feed additive had an effect on total egg weight and the effect began to be seen in treatment at P1 with a concentration of 2.5 g/Kg. The average total egg weight at P1, P2, P3, and P4 was significantly different and higher than the control. The total weight of duck eggs in the nanochitosan treatment of 10 g/kg feed weight was higher than the nanochitosan treatment at levels of 2.5; 5, and 7.5 g/kg feed weight.

Figure 2. Morphology of duck egg after treatment with nanochitosan feed additive for 4 weeks

The egg weight of the wading ducks increased after the addition of nanochitosan at 2.5; 5; 7.5; and 10 g/kg feed weight. Variable increase in value is influenced by various kinds of nutrients in the egg and water molecules that contribute to the increase in the weight of egg white, egg yolk, and eggshell. The weight of egg whites can be affected by the protein and air content in the egg whites. The weight of the yolk can be affected by the presence of fat, cholesterol, protein, and air in the yolk. Kirwan *et al*. (2021) stated that protein is the main component of eggs so that protein consumption will affect egg weight. Frita *et al*. (2017) stated that eggs with a lot of albumen and thick will result in an increase in egg weight.

Egg yolks affect the total weight of the eggs produced. The total weight of the egg is greater if the yolk formed is greater. Li *et al*. (2023) stated that nanochitosan is a cationic polymer that is insoluble in water, so that the water content can run normally during the egg formation process. Total egg weight is also affected by the presence of shells in the egg. Sulaiman *et al*. (2023) stated that eggshell weight ranges from 9-12% of the total egg weight. Nanochitosan is able to increase protein digestibility which can affect the absorption of calcium used in the formation of eggshells. Suthama *et al*. (2021) stated that protein digestibility affects the weight of calcium in eggs because it plays a role in calcium absorption in the presence of Ca binding protein (CaBP) or calcium binding protein which functions in transporting calcium to intestinal mucosal cells which are then circulated to the reproductive tract through blood vessels for egg shell formation.

The novelty of this research is about the use of nanochitosan as a feed additive to improve egg quality, especially the effect of nanochitosan to increase egg white protein content, egg yolk cholesterol content, and egg weight in wading ducks. This research is expected to open up opportunities for further research in the future on the effect of nanochitosan as a feed additive in improving egg quality in various other types of poultry. The results of this research also provide important information and knowledge about the biological effects of nanochitosan on laying ducks thereby increasing exploration in the use of nanochitosan products. This research can provide a reference for farmers regarding the levels of using nanochitosan as a feed additive in improving the quality of duck eggs.

CONCLUSION

Nanochitosan can be used as a feed additive to improve the chemical quality of eggs based on indications of increased protein content, cholesterol levels, egg white weight, egg yolk weight and duck egg weight. Nanochitosan as a feed additive with levels of 7.5 and 10 g/kg feed weight can be given to improve the chemical quality of duck eggs, especially in Pengging ducks, and in general in other local ducks.

It is necessary to conduct development research on the use of nanochitosan as a feed additive in various local ducks in Indonesia.

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